LIFELINE RESEARCH MEETING ABSTRACTS

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HORIZONS OF ATHEROSCLEROSIS RESEARCH AND THERAPY

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The treatment of coronary artery disease has witnessed a revolution in the last quarter century. We have mastered the diagnosis of coronary stenoses and cardiac ischemia. We have at hand an array of extremely effective therapies for treating ischemia, including medical therapy, and percutaneous and surgical revascularization. Yet, these modalities do not prolong life or prevent myocardial infarction except in selected subgroups. With regard to coronary risk reduction, we have also made considerable progress. Data from multiple clinical trials with LDL lowering agents have fully vindicated the "cholesterol hypothesis," documenting a reduction in coronary events and reducing all-cause mortality. Yet, even the most effective current therapies fail to prevent the majority of coronary events, and most patients with atherosclerosis have LDL levels in the "average" range. Thus, much remains to be done to combat the residual burden of atherosclerotic disease. In preparation for this task, we need to take into account shifting demographics and the emerging therapeutic targets. We will witness manifold demographic changes in coronary disease the next 20 years. The most important will be Globalization, Aging, and increasing adiposity and hence, the insulin resistance syndrome. New therapeutic opportunities will include aspects of dyslipidemias beyond LDL, including strategies to manipulate HDL (eg, Apo A-1, CETP, SRB-1, ABC-1), and "diabetic dyslipidemia" (PPAR-alpha agonists and insulin sensitizers including the PPAR-gamma agonists). Novel lipid-directed therapeutic targets also include cholesterol absorption and ACAT inhibitors. Aside from strategies targeting lipids, we will need to seek more information about nontraditional risk factors and their treatment, including antioxidants, Lp(a), homocysteine, and infectious agents. Although we can look back with some satisfaction on the advances of recent years in treatment of atherosclerosis, we must not become complacent, but pursue new avenues to limit even further the worldwide spread of this major cause of illness and death.

For the widest dissemination possible, these abstracts also appear on the Web at www.jvir.com in conjunction with the July issue of the *Journal of Vascular and Interventional Radiology* (published by Lippincott, Williams & Wilkins).

VEGF–INDUCTION OF ANGIOGENESIS

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The therapeutic implications of angiogenic growth factors were identified by the pioneering work of Folkman and colleagues over two decades ago.[1] Their work documented the extent to which tumor development was dependent upon neovascularization and suggested that this relationship might involve angiogenic growth factors that were specific for neoplasms. Subsequent investigations have established the feasibility of using recombinant formulations of such angiogenic growth factors to expedite and/or augment collateral artery development in animal models of myocardial and hindlimb ischemia. This novel strategy for the treatment of vascular insufficiency was termed *therapeutic angiogenesis.*[2] More recent data suggest that the basis for native as well as therapeutic neovascularization is not restricted to angiogenesis, but includes postnatal vasculogenesis as well. Data supporting these notions as well as derivative concepts and concerns are the subject of this Perspective.

Therapeutic angiogenesis

Preclinical studies established that angiogenic growth factors could promote collateral artery development in animal models of peripheral and myocardial ischemia (reviewed in [3]). Morphometric analyses documented that such enhanced vascularity encompassed a range of vessel caliber, from medi-

um-sized arteries visualized by premortem angiography to increased capillary density demonstrated in postmortem histology. The median range of new vessel growth, however, appears skewed to smaller caliber arteries less than 180 μ in diameter. Indeed, there is good reason to suspect that a proportion of newly recognized medium-sized arteries may develop as a result of "arteriogenesis" (ie, in situ proliferation of preexisting arteriolar connections into larger collateral vessels).[4] Whether such remodeling occurs as a direct result of growth factor modulation or as a flow-mediated response to augmented downstream capacitance remains to be determined.

The angiogenic growth factors employed in these preclinical studies have been administered as recombinant protein or by gene transfer, and include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)–1, FGF-2, and hepatocyte growth factor (HGF). More recently, a drug—the angiotensin-converting enzyme (ACE) inhibitor, quinapralat[5]—and naked DNA encoding for the transcription factor hypoxia inducible factor–1 (HIF-1)[6] have each been shown to enhance neovascularization in the rabbit ischemic hindlimb model.

The optimal preparation and delivery strategy for therapeutic neovascularization is the subject of ongoing clinical investigation. The potential requirement to maintain a suitably high and local concentration over a period of days to weeks constitutes an advantage for gene transfer versus recombinant protein therapy. While viral vectors[7] may enhance transfection efficiency and thus yield higher levels of gene expression, this may be less relevant to strategies in which gene products such as VEGF include a signal sequence, which permits active secretion from intact cells; previous studies from our laboratory[8] have documented that naked DNA that encodes for a secreted protein—as opposed to proteins that remain intracellular—can yield meaningful biological outcomes because of paracrine effects of the secreted gene product.

Proof of concept for the notion that therapeutic angiogenesis could be successfully extended to human subjects was first demonstrated using gene transfer of naked DNA encoding for VEGF (phVEGF) for the treatment of critical limb ischemia.[9] Treatment was initiated with 100 mg of phVEGF using a dose-escalating design. Three patients presenting with rest pain (but no gangrene) and treated with 1000 mg were subsequently shown at 1-year follow-up to have improved blood flow to the ischemic limb and remain free of rest pain. With the increase in dose of phVEGF165 to 2000 μg angiographic and histologic evidence of new blood vessel formation became apparent.[9] More recently, the use of intramuscular gene transfer, employed initially as a means of treating patients in whom vascular disease in the ischemic limb was too extensive to permit an intra-arterial approach, achieved marked improvement in collateral vessel development in patients with critical limb ischemia.[10] Objective findings of bioactivity in this preliminary report included improvement in the ankle-brachial index, angiographic evidence of newly visible collateral blood vessels, and demonstration by magnetic resonance angiography of improved lower extremity blood flow. Ischemic ulcers healed or markedly improved in four of seven limbs, including successful limb salvage in three patients recommended for below-knee amputation.

Successful application of both gene transfer and recombinant protein administration for the treatment of myocardial ischemia in human subjects was reported in 1998. The former involved direct intramyocardial injection of phVEGF as sole therapy for myocardial ischemia refractory to conventional therapy.[11] Among 24 consecutive patients treated with this strategy to date, anginal episodes requiring sublingual nitroglycerin were reduced from nearly 60 per week to less than three per week. Objective evidence of improved perfusion was documented by a near doubling of treadmill exercise time, and improved myocardial blood flow on stress as well as resting nuclear perfusion scans. The improvement in perfusion observed at rest is consistent with resolution of hibernating myocardium, a finding that has been recently confirmed using catheter-based electromechanical mapping.[12] Recombinant protein administration using FGF-1 has also been reported to augment myocardial revascularization and improve functional status in patients undergoing concurrent coronary artery bypass surgery.[13]

In vitro studies have suggested certain mechanisms that may have contributed to the apparent benefit as well as safety of phVEGF gene transfer in these early trials. While ECs were previously viewed solely as the target for VEGF, it is now clear that ECs subjected to hypoxia can synthesize VEGF as well.[14] This autocrine feature of VEGF creates the opportunity for amplifying the effects of even a small amount of exogenous VEGF, as EC proliferation in the ischemic territory creates additional potential cellular sources of VEGF synthesis and secretion. Moreover, the recent observation that VEGF may upregulate its own (VEGFR-2 or KDR) receptor[15] establishes a second basis for autocrine and paracrine amplification.

VEGF has also been shown to inhibit EC apoptosis by activating the serine-threonine protein kinase Akt through a process requiring integrin ligation.[16] This finding suggests a mechanism other than mitogenesis by which a net increase in EC viability may be accomplished. Given the limited twofold to fourfold increase shown for VEGF on cellular proliferation, it is possible that the contribution of enhanced EC survival under conditions of severe ischemia is critical to the proangiogenic effects of VEGF and other angiogenic growth factors.

The preclinical and clinical studies of therapeutic angiogenesis performed to date have repeatedly shown that VEGF-induced angiogenesis is not indiscriminate or widespread, but is instead restricted to sites of ischemia. This appears to result from paracrine upregulation of the principal high-affinity VEGFR-2 (KDR) receptor in response to factors released from hypoxic skeletal myocytes.[17] Receptor upregulation on ECs within the region of lower limb or myocardial ischemia thus enables these cells to act as magnets for any VEGF secreted into the ischemic milieu. Only when VEGF expression is locally protracted at high levels has it been possible to violate this principle.[18]

Risk factors for neovascularization

Preliminary clinical findings in patients with critical limb ischemia indicated that the response to phVEGF gene transfer was most robust and expeditious in young patients with premature atherosclerosis involving the lower extremities, so-called Buerger's disease.[19] This clinical observation was supported by experiments performed in live animal models, specifically young (4-5 years) versus old (6-8 months) rabbits and young (8 weeks) versus old (2 years) mice. In both cases, native neovascularization of the ischemic hindlimb was markedly retarded in old versus young animals. Retardation of neovascularization in old animals appeared in part to result from reduced expression of VEGF in tissue sections harvested from the ischemic limb.[20] Similarly retarded neovascularization and reduced VEGF expression were observed in diabetic (NOD)[21] and hypercholesterolemic (ApoE-/-) mice.[22] Cell-specific immunostaining localized VEGF protein expression to skeletal myocytes and infiltrating T cells in the ischemic limbs of C57 mice; in contrast, VEGF-expressing T cell infiltrates were found to be severely reduced in ischemic limbs of mice in which angiogenesis was impaired. Transendothelial migration of human T cells has been previously shown to be compromised in elderly versus young subjects, although the basis for this defect in transmigration remains enigmatic.[23] The critical contribution of T cells to VEGF expression and collateral vessel growth has been reinforced by the finding of accelerated limb necrosis in athymic nude mice with operatively induced hindlimb ischemia.[22]

Reduction in endogenous VEGF expression, however, was not the only factor contributing to impaired neovascularization in these animals; older, diabetic, and hypercholesterolemic animals—like patients—also exhibit age-related endothelial dysfunction, manifest as reduced vasodilation and decreased production of NO in response to endothelium-dependent agonists.[20] Endothelial dysfunction did not preclude a favorable response to cytokine replacement therapy; indeed, the absolute magnitude by which blood pressure ratio, angiographic score, and capillary density were increased in response to supplemental administration of recombinant VEGF protein was similar for young and old animals. In older animals, however, these indices failed to reach the ultimate levels recorded in younger animals, apparently reflecting the inherent limitations imposed by a less responsive EC substrate.

This clinical experience and these animal studies have two implications. First, the findings suggest that the fundamental mechanism by which therapeutic neovascularization augments collateral development is to provide cytokine supplements to individuals who, because of advanced age, diabetes, hypercholesterolemia, and/or other as yet undefined circumstances, are unable to appropriately upregulate cytokine expression in response to tissue ischemia. In this regard, ligand supplementation may be analogous to erythropoietin administration in patients with refractory anemia.

Second, cytokine administration clearly comprises only one aspect of the therapeutic intervention. Regardless of how much ligand is administered, the resident population of ECs that is competent to respond to an available level of angiogenic growth factors may also constitute a potentially limiting factor in strategies designed to promote neovascularization of ischemic tissues. A reasonable goal may therefore consist of developing a complementary strategy that would provide substrate together with ligand, a "supply side" version of therapeutic neovascularization.[24]

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DELIVERY OF FGF-FAMILY PEPTIDES FOR NEOVASCULARIZATION

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The idea that we might be able to control selectively the formation of blood vessels keeps attracting investigators and investors as well: suppress angiogenesis and thus inhibit the growth of tumors, or provoke it and thus revascularize ischemic tissues, and two major killers, cancer and atherosclerosis, are eliminated! While this is clearly a naive oversimplification, it is still very impressive to observe new blood vessels reach toward a corneal pocket containing a piece of tumor or a pellet slowly releasing FGF in the best established in vivo angiogenesis test. Many of the other tests cover at least some partial aspects of angiogenesis such as endothelial cell migration, proliferation, enzymatic activity, or tube formation, and representative members of the FGF family are positive in all of these assays. Having FGF as a defined peptide rather than an obscure extract and knowing much about its angiogenic and other properties, why has there not been more sustainable progress applying this (and many other "growth" factors) for therapeutic neovascularization in vivo?

While it is impossible to predict all interactions of a new therapeutic agent in vivo, some fundamental rules observed in tissue culture should not be overlooked. For example, FGF-I needs to be applied to